MATERIAL AND METHODS

Telomerase measurement by Q-PCR

Protein was measured by the RC DC Protein Assay by BioRad (Hercules, CA). All the reactions were performed in a Rotor-Gene 3000 thermal cycler (Corbett Research, Sydney, Australia) in a final volume of 20µl. Each reaction included: 1X PCR buffer (Invitrogen, Carlsbad, CA), 1.5mM $MgCl_2$, 0.2mM dNTPs, 0.4X SybrGreen (Molecular Probes, Eugene OR), 1% DMSO, 1u of Platinum Taq (Invitrogen, Carlsbad, CA), 1µl of 5µM TSC primer (AATCCGTCGAGCCGAGTT), 2µl of 3µM tel2b primer (GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT) and 240ng of protein. The tel2b primer was previously designed for amplification of telomeric DNA (1) and the TSC primer is a modification of the TS primer used in the TRAP assay. The reactions were incubated at 30°C for 30 minutes, denatured at 95°C for 15 minutes, and amplified in 50 cycles of 95°C for 15 seconds, 50°C for 30 seconds, and 72°C for 60 seconds. All the samples were run in triplicates and the median value was used for calculations. For each sample, a heat inactivated negative control was created by incubating the protein extract at 80°C for 10 minutes. Each experiment included a four point standard curve (2-fold serial dilutions from 10 to 1.25ng of protein) generated with an hTERT transduced cell line (QhTERT) (2). The standard curve permitted transformation of the samples Cts into equivalent nanograms of QhTERT protein. For each sample telomerase activity was expressed as the percentage relative to the activity of 10ng of QhTERT protein. These values were transformed to log 10 for all subsequent calculations. Three positive controls were included in all reactions; one of the controls was used to normalize between experiments.

γ-H2AX and Dec1 immunostaining

Paraffin-embedded, lightly paraformaldehyde-fixed slides were processed using a modification of previous protocols (3, 4). Slides were incubated in 10 mM sodium citrate pH 6.5 for 40 minutes at 90^{o}C for antigen retrieval. Slides were blocked in 3% BSA (Sigma) and incubated at 4^{o}C overnight with a mouse monoclonal anti-phospho-histone H2AX antibody (Ser139) clone JBW301 (Upstate Biotech, Charlottesville, VA) diluted 1:1000 in blocking buffer and rabbit anti-Dec1 (a generous gift from Dr. Adrian Harris) diluted 1:1000 in blocking buffer. Slides were washed with 5% goat serum/PBS and incubated with 1:1000 dilution of secondary antibodies (goat anti-mouse Alexa Fluor 568 IgG_1; goat anti-rabbit Alexa Fluor 647 IgG; Molecular Probes, Carlsbad, CA). Antibodies were fixed with 4% paraformaldehyde for 20 minutes followed by 0.25 mM glycine for 20 minutes. Nuclear DNA was counterstained with 10 mg/ml DAPI. Slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA). Images were taken on a Zeiss LSM Meta 510 microscope at 63X with excitation at 543 nm for Alexa 568, 633 nm for Alexa 647, and 405 nm for DAPI, using sequential scans at constant settings for all slides. Images were quantitatively analyzed by calculating the average γ-H2AX and Dec1 staining intensity of at least 100 nuclei per biopsy, in a minimum of 3 fields (5, 6).

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3. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). Mol Cell 2004;14:501-13.
4. O'Sullivan JN, Bronner MP, Brentnall TA, et al. Chromosomal instability in ulcerative colitis is related to telomere shortening. Nat Genet 2002;32:280-4.
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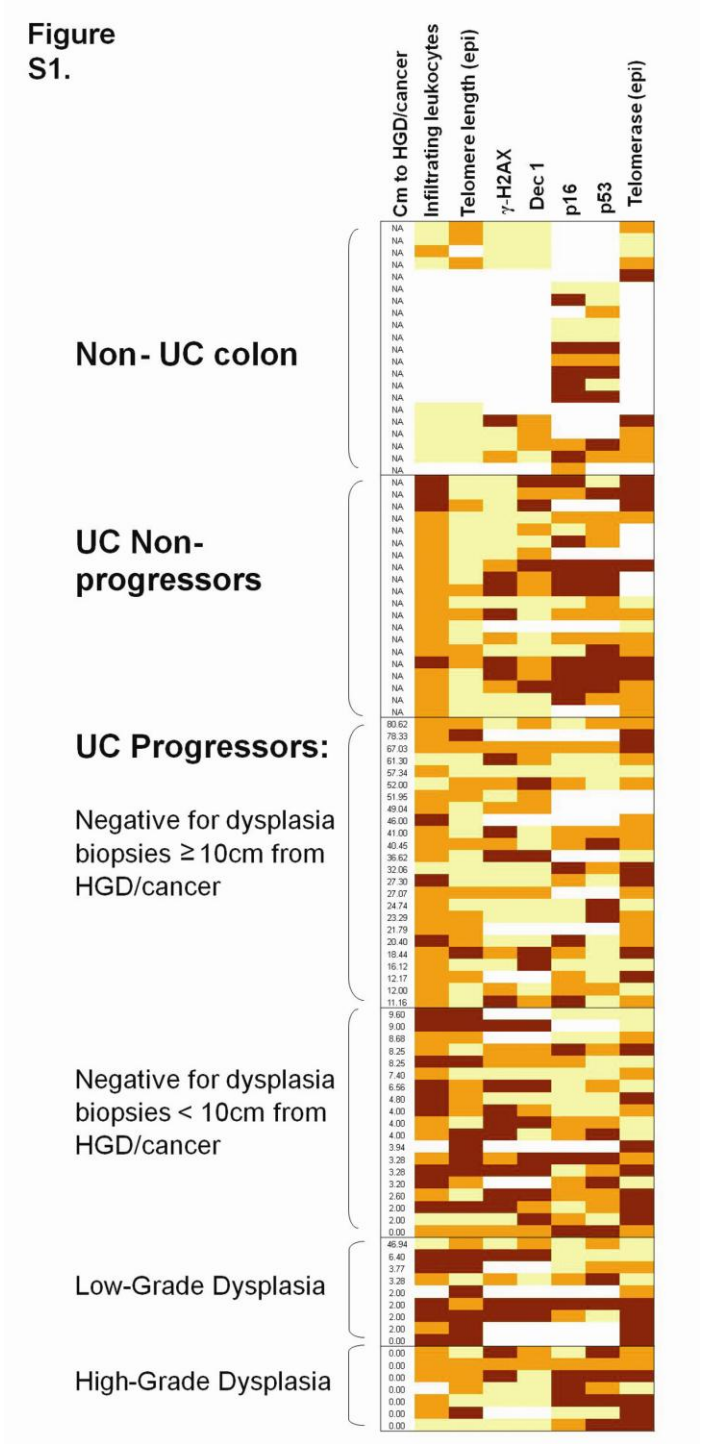

Figure S1. Heat map of infiltrating leukocytes and alterations for epithelial telomere length, γ-H2AX, Dec1, p16, p53, telomerase. Biopsies are represented by horizontal lines and are grouped by histology. Distance (in cm) from HGD or cancer is indicated. The level of alteration is color coded such that darker colors indicate higher levels of alterations. Epithelial telomere length: yellow- long telomeres, orange- medium telomeres, brown- short telomeres, based on tertiles. γ-H2AX, Dec1, p16, p53, and telomerase: yellow- low, orange- medium, brown- high, based on tertiles.

Figure S2.

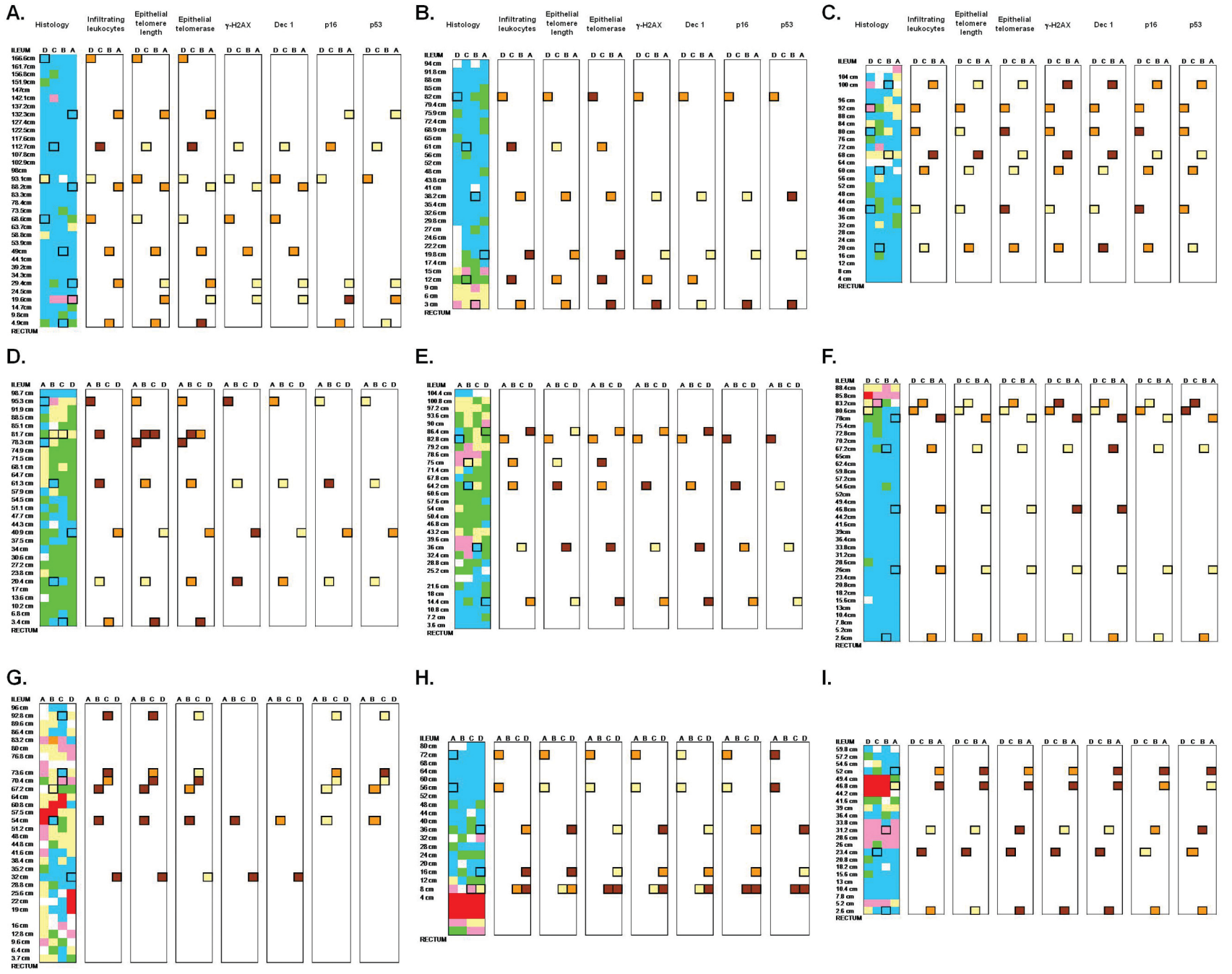
A.
B.
C.
D.
E.
F.
G.
H.
I.
Histology
Infiltrating leukocytes
Epithelial telomere length
Epithelial telomerase
γ-H2AX
Dec 1
p16
p53
ILEUM
RECTUM

Figure S2. Colon maps of Progressors. (A, B, C, D, E) patients with HGD. (F, G, H, I) patients with cancer. The cm on the left indicate distance from rectum. The first column shows a color-coded histology map including all biopsies sampled at colectomy: blue-negative for dysplasia, green-indefinite, yellow- LGD, pink- HGD, red- cancer. The bold squares indicate the biopsies analyzed in this study. The columns to the right of the histology map show the most informative parameters measured in this study, color-coded for the level of alteration. Lamina propria infiltrating leukocytes: yellow- 1+, orange- 2+, brown- 3+. Epithelial telomere length shows three levels based on tertiles: yellow- long telomeres, orange- medium telomeres, brown- short telomeres. Epithelial telomerase, γ-H2AX, and Dec1 show three levels based on tertiles: yellow- low amount, orange- medium amount, brown- high amount.

Supplementary Table 1. Statistical significance of senescence parameters differences between stages of UC progression

	Telomere length epithelium	Telomere length stroma	Telomerase epithelium	Telomerase stroma	γ-H2AX	Dec1
normal vs. NP	0.416	0.043	0.086	0.792	0.832	0.524
NP vs. P neg≥10cm	**0.007**	0.850	**0.040**	0.761	0.933	0.465
P neg≥10cm vs. P neg <10cm	**0.022**	0.922	0.509	0.696	**0.046**	**0.016**
P neg<10cm vs. LGD	0.147	0.980	1.000	0.431	0.827	0.694
LGD vs. HGD	**0.039**	0.368	0.315	0.958	0.361	**0.045**
NP vs. P neg	**<0.0001**	0.669	0.059	0.865	0.370	0.710
all compared (Kruskal-Wallis)	**<0.0001**	0.279	0.220	0.775	0.187	0.051

p-values except last row correspond to Mann-Whitney 2-tailed tests

NP: Non-Progressors; P: Progressors; neg≥10cm: negative for dysplasia located ≥ 10cm from HGD or cancer; neg <10cm: negative for dysplasia located <10cm from HGD or cancer; LGD: Low-Grade Dysplasia; HGD: High-Grade Dysplasia.